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make should make BSK11 vector of HindIII (blunt) / HindIII to clone in
full length 5.2 kb PSE.
do a double digest
EIP
prepare for various

I should also make an XbaI vector of BSK11 +

p15.81 a	25 µl BSK11 + p4.11 ✓	p15.81 b	25 µl BSK11 + p4.11
	10 µl NEB 2 ✓		10 µl NEB 2 ✓
	55 µl H ₂ O ✓		10 µl 10x BSA ✓
	5 µl HindIII ✓		50 µl H ₂ O ✓
	5 µl HindIII ✓		5 µl XbaI ✓
	To 3:15 P.M.		

I have become interested in designing an Adenovirus (say Ad5) that can only replicate in prostate + PSA cells e.g. LNCaP. If such a virus could be made e.g. EIA driven by the PSA enhancer we could have something quite marvelous. It would be a tissue-specific virus.

One approach would be to clone EIA w/ PSE, and gene virus first in 293 as a replication competent virus. purify virus and infect LNCaP - get a productive infection. Now virus would only grow in 293 if LNCaP w/ added hormone, R1881; And in no other cell.

The mutant could be made to have a unique restriction site to easily identify amongst plaques, a PCR of internal PSE sequences.

What is the nature of the EIA enhancer/promoter in Ad5?
Can it be functionally replaced w/ minimal PSE?

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Date

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To pull out p-gal out w/ XbaI. To clean the vector use
BspHI, ScaI, XbaI or NaeI, then p-gal
runs as the upper single band.

I have not yet tested dropping the vector w/ the
enzymes listed above.

if we wanted to make a smaller CAT we could clone it into
NotI sites. This drops ~ 700 bp of SV40 out
of CAT. But our CAT is already small enough
for Adeno.

Back to p-gal. We should put in the BglII/HindIII piece then
the minimal enhancer, then p-gal. Could PCR the
BglII minimal enhancer - promoter f. have two
cloning sites.

AGENDA

- I. SAB Meeting - Post-mortem
changes in emphasis
changes in deployment of personnel
- II. Construction of Clones - Eric and Dan
Within XbaI-ApaI - characterize and transfer to CAT construct - Eric
and Joe
Internal deletion mutants within PSE CAT - CN13 and CN42
NdeI/Bgl II 1861 bp
StuI/Bgl II 1421 bp
Cla I deletion
Minimal enhancer driving β -gal - Det
XbaI - 5729 = deletion
- III. Transfection of PSE clones in LNCaP - Eric
- IV. Tissue Specificity of Minimal Enhancer
LNCaP - AR mutation
- V. Construction of Adenovirus vector
Cotransfection
- VI. Animal Tissue Work-up - Gail
Hand grind (Hand) vs. Tissue-Tearer (TT) Can use TT if carefully, worse
Protein Concentration
Protease Inhibitors
Decision vs PCR and rtPCR
tubes, look for other tubes
- VII. Liposomes - Henry, Gail and Lena
DOTMA and DOPE liposomes
Formulation
Charge-Ratio, Osmolarity, Gel-Electrophoresis,
Trip to INEX and Peter Cullis - Henry
- VIII. Direct Injection of DNA into Tumors - Eric
Technique of Intra-tumor Injection
Assay by CAT, β -gal, histology?
Histology Lab source in Concord
Help from Dermatology

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Lis - dermatology

F. Sykes - short blonde hair

Proceeded w/

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(cont.)

I also continued the thoughts on a prostate-specific replication competent Ad5. Ad5 is not oncogenic, infects essentially all cell-types. If I recall correctly, Adeno binds to an ICAM as its cell surface receptor.

The notion continues to replace the promoter/enhancer of EIA w/ the minimal PSE. Then to grow in 293 or C127 to get replication competent virus.

Things seem a biological containment. WE Adeno5 is more dangerous than a prostate-specific Adeno. Indeed we are narrowing the host range to prostate cells. Then dose should be important as well as the route of delivery. While that containing and handling of Adeno may be enough containment it is also easy enough to put in HSV-tk gene driven by either the minimal PSE enhancer or enhancer/promoter responsive to EIA binding.

Now the virus could be shut down by acyclovir. aif. It is positively contained. This would probably fly for IV injection.

Does the Adeno5 DNA polymerase incorporate the defective ~~thymidine~~ guanine triphosphate nucle from acyclovir? HSV-tk? unknown.

I talked w/ Jonathan Simone today & she has data that says HSV-tk and acyclovir does not inhibit Adeno - at least the plate was wiped out.

- But would it have been wiped out anyway? Isn't that what HSV-tk will do if infection is going on. What would we expect w/ HSV-tk in an replication defective Adeno.

- search on Adeno DNA polymerase - ask Joe Newell.
has anyone purified the enzyme.

how about cytosine arabinoside

I talked w/ Jonathan about this idea & he liked it. I hope he doesn't spread the idea. I think it promising & somewhat revolutionary. - on the engineered tissue-specific virus for therapy.

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